

## Effects of simulated acid rain on the allelopathic potential of invasive weed *Wedelia trilobata*

RUILONG WANG<sup>1</sup>, SHAFIQ UR REHMAN<sup>1</sup>, XIAOTING LIANG<sup>1</sup>, YUANYUAN SONG<sup>1</sup>, YIJUAN SU<sup>1</sup>, SCOTT R BAERSON<sup>2</sup> and RENSEN ZENG<sup>1\*</sup>

State Key Laboratory for Conservation and Utilization of Subtropical Agro-bioresources  
South China Agricultural University, Guangzhou 510642, China  
E. Mail: rlw2009@scau.edu.cn , rszeng@scau.edu.cn

(Received in revised form: April 1, 2012)

### ABSTRACT

Acid rain poses a major threat to natural ecosystems in rapidly-developing industrialized regions like southern China. Despite the significant environmental impact of acid rain, little is known about its effects on important aspects of ecosystem dynamics such as plant-plant allelopathic interactions. The major invasive weed *Wedelia trilobata* in southern China, was used in this study to examine the possible effects of acid rain on the allelopathic potential of invasive plant species. The phytotoxicities of aqueous leachates and dried leaf litter of field-grown *W. trilobata* plants exposed to simulated acid rain (SAR) of pH 2.5, 4.0, 5.6, 7.0 water control] were determined in *in-vitro* assays on two receptor species: *Brassica campestris* and *Raphanus sativus*. Substantial increases in the phytotoxicity of the leachates as well as leaf litter were observed as a function of decreasing SAR pH. Additionally, glasshouse experiments were done to determine the effects of various SAR-treatments on *W. trilobata* biomass accumulation and shoot height, both parameters showed modest increases at SAR pH 4.0 and decreases at SAR pH 2.5 than control (pH 7.0) plants. These data indicated that acidic conditions increased the allelopathic potential of *W. trilobata*, suggesting that acid rain exposure may increase the invasiveness of this weed.

**Key words:** Allelopathy, biological invasion, *Brassica campestris*, phytotoxicity, *Raphanus sativus*, simulated acid rain, *Wedelia trilobata*

### INTRODUCTION

In recent years, acid rain has received less attention in developed nations, yet it remains a major problem to the environmental and agricultural concerns of developing nations worldwide (10,27,37). Currently approximately 30% of total territory of China is seriously affected by acid rain, mainly in the highly-industrialized central, eastern and southern regions (7,17,37). The prolonged exposure of large geographical areas in southern China to acid rain has caused enormous economic losses and substantial damage to local ecosystems (37).

Plant-plant allelopathic interactions, involving the chemical inhibition of one plant species by another, represents a form of chemical warfare between the neighboring plants competing for growth resources: light, water, and nutrients (6,26,31). Allelopathic

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\*Correspondence author, <sup>1</sup>Key Laboratory of Tropical Agro-environment, Ministry of Agriculture, South China Agricultural University, Guangzhou 510642, China, <sup>2</sup>USDA-ARS, Natural Products Utilization Research Unit, P. O. Box 8048, University, MS, 38677, USA.

plants, particularly non-native invasive species may exert profound effects on the structure, composition and evolution of native plant communities through loss of susceptible species and imposition of selective pressure favouring the individuals plants resistance to allelochemicals inhibition (3,5,13,16). It is known that unfavorable environmental conditions enhances the potency of allelochemical mixtures released as leachates or exudates into the soil (4,6,23). It is therefore reasonable to speculate that conditions of increased acidity due to exposure to acid rain could similarly enhance the plant allelopathic potential in specific cases, thus increasing the likelihood of allelopathic weeds infestations.

*Wedelia trilobata* L., a perennial weed originating from tropical Central and South America, is now most noxious invasive plant species in southern China (32). Its seed production is very low, but the plant spreads quickly through vegetative propagation, with new plants arising from its nodes (34,35). The aggressive growth habit, tolerance to environmental stresses, and capacity to synthesize allelochemicals have all contributed to the ability of *W. trilobata* to rapidly invade and cause significant damage to natural ecosystems and plantations in southern China (33,35).

Despite the potential for adverse environmental conditions such as acid rain to enhance the allelopathic potential and/or competitiveness of certain invasive weeds, relatively few studies have addressed this question. This study aimed to compare the growth and allelopathic potential of *W. trilobata* seedlings exposed to simulated acid rain (SAR) at various pH values.

## METHODS AND MATERIALS

### Materials and acid rain preparation

*W. trilobata* plants were collected in May, 2010 from Research Farm field, South China Agriculture University, Guangzhou (N 23°16', E 113°34'). Cuttings (7 cm long) were prepared and transplanted into plastic pots (9 cm dia, 12.5 cm height) containing 400 g soil. The plants were then maintained in glasshouse (25 ± 1°C, 14/10 h day/night cycle, 75% relative humidity).

SAR solutions used for treatment of field- and glasshouse-maintained *W. trilobata* plants were prepared by first combining 1 M H<sub>2</sub>SO<sub>4</sub> and 1 M HNO<sub>3</sub> in a ratio of 2.5:1 (v/v), to provide an anion composition similar to natural rainfall typically occurring in the vicinity of Guangzhou, China (7). SAR solutions (pH 2.5, 4.0 and 5.6) were then prepared from the H<sub>2</sub>SO<sub>4</sub>/HNO<sub>3</sub> mixture by adding distilled water (10,37). Control was distilled water (pH 7.0).

### Glasshouse SAR foliar application experiments

When the plants of *W. trilobata* were approximately 10 cm in height, 20-pots containing plants of uniform growth were selected and randomly divided into four groups. Each group of plants was treated with SAR (pH 2.5, pH 4.0, pH 5.6, 7.0 control distilled water). The SAR treatments were applied twice weekly using irrigation sprinklers delivering droplets (approx. 0.4 mm dia), with 32.5 ml of each solution applied per week (7,10). The plants were moved randomly twice a week to avoid internal glasshouse effects and then harvested after 45 days. Treatments were replicates five times. The height of each

plant was recorded and plants were partitioned into leaves, stems and roots. After drying at 70°C for 72 h, the leaves, stems, and roots of each plant were weighed.

### Bioassays

The bioassays were conducted to determine the effects of SAR on the allelopathic potential of *W. trilobata* leaf aqueous leachates and dried leaf litter. In September 2010, a field site (approximately 300 m<sup>2</sup>) was selected at Research Farm, South China Agriculture University which had exhibited highly favorable conditions for the growth of wild *W. trilobata* for two consecutive years. In this location *W. trilobata* had inhibited the growth of other plants and essentially existed as monoculture. The site was divided into 16 plots (1.5 × 1.5 m each), each of which was treated once daily for 3 d with 14 L of either SAR pH 2.5, 4.0 5.6 or distilled water (pH 7.0), using irrigation sprinklers as described above (7). Approximately 24 h after the final SAR treatment, the third and fourth leaves (counted basipetally from the apex) of the plants were harvested and used for the preparation of aqueous leachates and dried leaf litters tested in bioassays.

For aqueous leachate preparation, 50 g of freshly-collected leaves from each experimental plot were soaked in 100 mL distilled water for 48 h at 25 ± 1°C to get 0.5 g/mL aqueous leachates. The leachates were filtered through two layers of filter paper and then diluted with distilled water to prepare required concentrations of 0.5, 0.25 and 0.125 g/mL. The pH value of leachates was adjusted to 7.0 with 1 M NaOH or HCl.

*Brassica campestris* L. and *Raphanus sativus* L., commonly used in allelopathic studies (31), were chosen as the receptor species. Twenty germinated seeds of either *B. campestris* or *R. sativus* were sown on two layers of filter paper (9 cm dia, qualitative filter paper, No.1) in 9 cm dia glass Petri dishes containing 5 mL aqueous leachate prepared from SAR or control-treated *W. trilobata* leaves (0.5, 0.25 and 0.125 g/mL) or distilled water (control). The Petri dishes were then incubated in a growth chamber for 5 days (25 ± 1°C, 14/10 h day/night cycle, 75% relative humidity). After the 5 day incubation period, seedling shoot heights and root lengths were recorded. There were four independent replicates.

The allelopathic effects of *W. trilobata* dried leaf litter leachates were evaluated using the sandwich method (11,30). The fresh *W. trilobata* leaves collected from each SAR- and control-treated field plot were first air-dried, then either 10, 30 or 50 mg of the dried leaf material was placed between two layers of semi-solid agar (0.5% w/v) in 50 mL beakers. Five germinated *B. campestris* or *R. sativus* seeds were placed on the surface of the agar and the beakers were covered with parafilm and placed in a growth chamber for 3 days at 25 ± 1°C in dark. Petri dishes containing no dried leaf litter between the agar layers were used as control. After completion of the incubation period, seedling shoot heights and root lengths were recorded. All experiments were conducted with four independent replicates.

**Statistical analysis:** All data are presented as means ± standard error (SE). Significant differences ( $P < 0.05$ ) were analysed using one-way ANOVA followed by Duncan's multiple-range test using the SPSS 16.0 Software Package (SPSS, Inc., Chicago, IL, USA).

## RESULTS AND DISCUSSION

### *W. trilobata* symptomology following SAR exposure

In glasshouse conditions, exposure of *W. trilobata* plants to SAR caused visible damage to plants and the damage was more in young leaves. Leaf chlorosis spots on young leaves were observed in plants treated with pH 4.0 SAR at 16 days post-treatment. Similar symptoms appeared on plants treated with pH 2.5 SAR after 1-week. At 13 days post-treatment, plants exposed to pH 2.5 SAR also exhibited the necrotic lesions in young and older leaves, however, there was no lethality (severe reductions in growth). Plants treated with pH 5.6 SAR did not show adverse symptoms than control (pH 7.0). Overall, *W. trilobata* plants grown under these conditions were relatively tolerant to acidity of SAR treatments.

### *W. trilobata* growth

In glasshouse studies, the influence of exposure of *W. trilobata* plants to SARs [pH 2.5, 4.0, and 5.6, and control (pH 7.0)] were monitored on the plant height and dry matter accumulation (roots, total shoot and leaves). The plants exposed to SAR of pH 5.6 did not influence the plants height (Fig. 1), dry biomass accumulation in roots, shoots, and leaves than control (Fig. 2). Contrarily, the SAR (pH 4.0) modestly stimulated the growth, increased the plant height (13.1%) and total plant dry biomass (19.8%) relative to control (Fig. 1). The increase in total biomass with pH 4.0 SAR was largely due to the growth stimulation in leaves and shoots (Fig. 2). While the SAR (pH 2.5) inhibited the growth of *W. trilobata* and the decreased the plant height by 11.8% (Fig. 1) and the reduction in biomass in total biomass, root, shoot and leaf biomass was 18.1, 19.1, 15.6 and 19.9%, respectively, over the control plants.

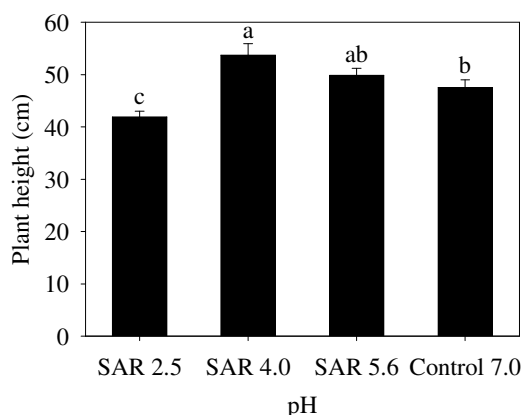


Figure 1. Effects of simulated acid rain (SAR) on *Wedelia trilobata* shoot height. *W. trilobata* plants were sprayed with SAR [pH 2.5, 4.0, 5.6 and 7.0 (control-distilled water)] treatments twice a week, and the shoot height was measured at 45 d after treatments. Values are means  $\pm$  SE from five replicates. Different letters indicated significant differences ( $P < 0.05$ ) according to Duncan's multiple range test.

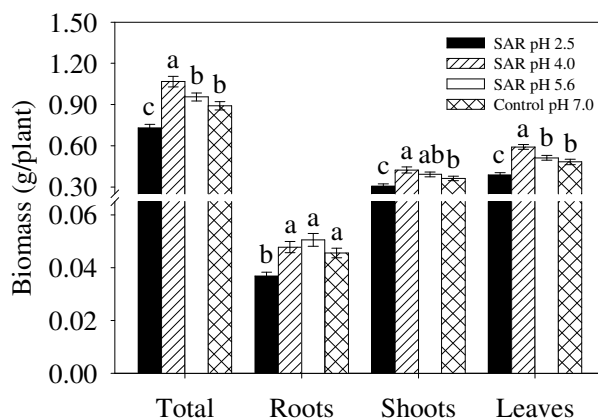


Figure 2. Effects of simulated acid rain (SAR) on biomass accumulation in roots, shoots, leaves of *Wedelia trilobata* at 45 d after treatments. The *W. trilobata* plants were sprayed with SAR [pH 2.5, 4.0, 5.6 and 7.0 (control-distilled water)] twice a week. Values are means  $\pm$  SE from five replicates. Significant differences ( $P < 0.05$  using Duncan's multiple range test) among treatments in a group are indicated by different letters above bars.

### *W. trilobata* allelopathic potential

The phytotoxicity of aqueous leachates prepared from the *W. trilobata* plants exposed to SAR (pH 2.5, 4.0, 5.6) or control (pH 7.0, distilled water) were first evaluated as inhibitory effects on the seedlings growth of *B. campestris* (Fig. 3A and C) and *R. sativus* (Fig. 3B and D). The leachates from SAR (pH 4.0 and 2.5)-treated *W. trilobata* plants significantly decreased the shoot height and root length of both test crops: *B. campestris* and *R. sativus* seedlings relative to control (pH 7.0) leachates. The inhibitory effects were greater for the SAR pH 2.5 leachate than the pH 4.0. The leachates of control (pH 7.0), SAR [pH 4.0, and SAR pH 2.5 undiluted (0.5 g/mL)] inhibited the root growth of *B. campestris* by 30.6, 37.6 and 50.5%, respectively, indicating a significant increase in *W. trilobata* aqueous leachates phytotoxicity as a function of increased SAR treatment acidity (Fig. 3C). The increase in the phytotoxicity of aqueous leachates of *W. trilobata* plants exposed to more acidic SAR treatments, indicated amelioration in plant allelopathic potential under acidic conditions (Fig. 3). However, the shoot height and root length of *B. campestris* and *R. sativus* seedlings grown in the presence of SAR pH 5.6 leachates were similar to control (pH 7.0). Apparent hormetic (stimulatory) effects are observed at the most dilute (0.125 g/mL) leachate concentrations, because the allelochemicals and other phytotoxins cause hormesis at sub-inhibitory concentrations (2).

Then 'Sandwich' bioassays (11,30) were done to evaluate the phytotoxicity of dried leaf litter obtained from the same SAR and control-treated *W. trilobata* leaf tissues used above to assess the aqueous leachate phytotoxicities (Fig. 4). The application of 10 mg leaf litter obtained from control (pH 7.0), SAR (pH 4.0) and SAR (pH 2.5) treated *W. trilobata* plants significantly decreased the shoot heights of *R. sativus* by 24.1, 46.8 and

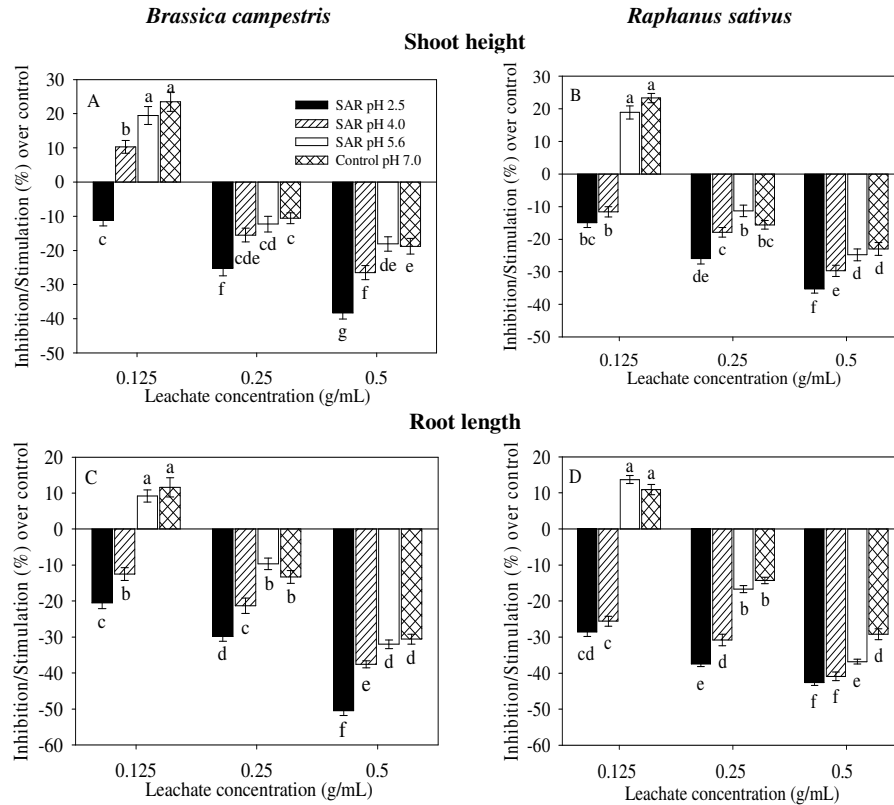


Figure 3. Phytotoxicity of *Wedelia trilobata* aqueous leaf leachates on shoot height and root length of *Brassica campestris* (A, C) and *Raphanus sativus* (B, D) at 45 d after treatments. Aqueous leachates prepared from leaves of *W. trilobata* plants sprayed with SAR [pH 2.5, 4.0, 5.6 and 7.0 (control-distilled water)]. Values are means  $\pm$  SE from four replicates. Significant differences ( $P < 0.05$  using Duncan's multiple range test) among all treatments are indicated by different letters above bars.

49.4%, respectively, indicating a significant increase in *W. trilobata* leaf litter phytotoxicity as a function of increased SAR treatment acidity (Fig. 4B). Similarly, root growth of *R. sativus* with 10 mg leaf litter from control (pH 7.0), SAR (pH 4.0), and SAR (pH 2.5) treatments was significantly inhibited by 28.6, 48.4 and 53.3%, respectively (Fig. 4D). The same trends were observed in shoot heights and root lengths of *B. campestris* (Fig. 4A and C). Shoot heights and root lengths of both receptor species grown in the presence of SAR pH 5.6 litters did not differ from the control (pH 7.0) values. The pH of all aqueous leachates was adjusted to 7.0 with 1 M NaOH or HCl. The aqueous leachates of *Wedelia trilobata* at 0.87 g FW/mL had no osmotic effects on seed germination and seedling growth (35, 36). The highest concentration used in this study was 0.5 g FW/mL.

Therefore the effects of pH and osmotic concentrations of SAR aqueous leachates can be excluded. The inhibitory effects observed in this study were due to phytotoxicity of leachates. *W. trilobata* is allelopathic to many crops (15,21,22,33,35). In *W. chinensis*, the allelochemicals trilobolide-6-O-isobutyrate and oxidoisotrlobolide-6-O-isobutyrate are identified (36). Later Zhang *et al.* found that trilobolide-6-O-isobutyrate and other five sesquiterpene lactones were responsible for the allelopathy of *W. trilobata* (38).

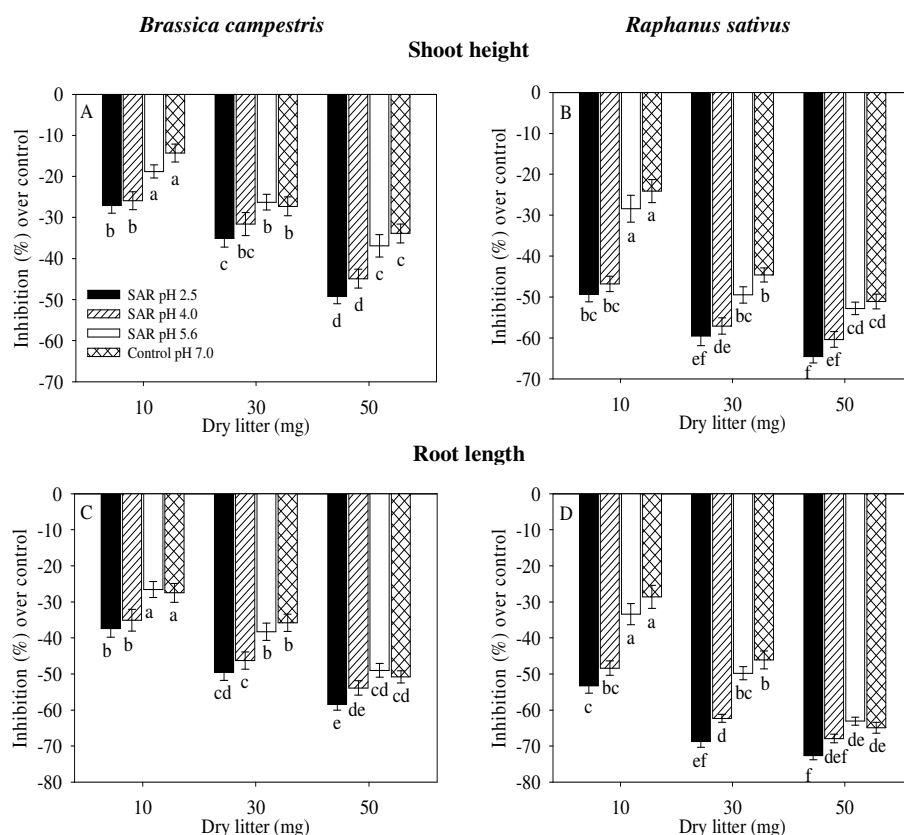


Figure 4. Phytotoxicity (Inhibitory effects) of *Wedelia trilobata* dried leaf litter on shoot height and root length of *Brassica campestris* (A, C) and *Raphanus sativus* (B, D) at 45 d after treatments. Aqueous leachates prepared from leaves of *W. trilobata* plants sprayed with SAR [pH 2.5, 4.0, 5.6 and 7.0 (control-distilled water)]. Values are means  $\pm$  SE from four replicates. Significant differences ( $P < 0.05$  using Duncan's multiple range test) among all treatments are indicated by different letters above bars.

Many studies have highlighted the deleterious effects of acid rain on plants, which impairs both vegetative and reproductive growth and development (8,12,19,24,29). In this study, only the most acidic SAR (pH 2.5) treatment reduced the plant height and dry

matter accumulation in *W. trilobata*. Thus *W. trilobata* is not tolerant to acidic conditions, although chlorotic lesions occurred in young leaves exposed to pH 4.0. The differences in susceptibility of younger versus older leaves to SAR may be partly due to the presence of tomentum (dense hairs mat) on the surface of older leaves, which intercepts and reduces the contact of SAR to leaf surface (9,20,28). The stimulation of *W. trilobata* growth with pH 4.0 SAR treatments and inhibition at lower pH values, is consistent with the responses to increasingly acidic SAR treatment pH values seen in other studies (1,9,14,18,25). The stimulatory effects of SAR (pH 3.5 and 4.5) has been attributed to the increased soil nitrogen content through the addition of  $\text{NO}_3^-$  from the SAR solutions, coupled with the tolerance of many plant species to such pH values (10,25).

The invasive alien weed *W. trilobata* has caused significant ecological damage in southern China, and much of this region is also exposed to frequent acid rains (7,15,36). The possibility raised by the present work that these two significant environmental threats could be interrelated is of considerable concern, and merits further investigation. Additional studies focusing on natural ecosystems will be required to fully elucidate, whether the acid rain facilitates the ecological invasions by *W. trilobata* and other invasive allelopathic plant species.

## CONCLUSIONS

The phytotoxicity of both aqueous leaf leachates and dry leaf litter from *W. trilobata* significantly increased following exposure to more acidic SAR (pH 4.0, pH 2.5; Fig. 3, Fig. 4) treatments. Its implication is that the allelopathic potential of *W. trilobata* and hence, its ability to invade and displace native plant species in natural ecosystems may increase in regions, where acid rain occur frequently. The observed increase in leachate phytotoxicity may be due to transcriptional or post-transcriptional up-regulation of allelochemical biosynthetic pathways, activation of stress-inducible pathways leading to the production of different, more potent allelochemicals, or structural and/or physiological changes caused by acidic conditions which facilitates the allelochemicals release (9,14,17).

## ACKNOWLEDGEMENTS

This research was financially supported by the National 973 Project of China (2011CB100400) and the Natural Science Foundation of China (31000260, 31070388, 31028018).

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